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Rate of Hepatitis B Virus Infection in Pregnant Women Determined by a Monoclonal Hepatitis B Surface Antigen Immunoassay

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The rate of HBsAg in 6,976 B-human chorionic gonadotropin (B-hCG)-positive specimens, as determined by the Auszyme Monoclonal assay (Abbott Laboratories, Abbott Park, Ill.), was 0.56% (39 of 6,986 repeatedly reactive [RR] and confirmed-positive specimens). All RR and confirmed specimens were hepatitis B virus positive by at least one additional test, yielding an assay specificity of 99.96%. The findings argue against unique attributes in the pregnant population that might produce inaccurate assay results.

An estimated 20,000 infants are born to HBsAg-positive women in the United States each year (3). Because these infants are at high risk of perinatal hepatitis B virus (HBV) infection, chronic HBV infection, and chronic liver disease, the American College of Obstetricians and Gynecologists, the American Academy of Family Practices, the American Academy of Pediatrics, and the Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices have recommended that all pregnant women undergo testing for HBsAg prior to delivery (2, 3).

The objective of this study was to examine the rate of HBV infection in specimens from pregnant females using the Auszyme Monoclonal assay. We investigated whether pregnancy had any potential influence on the specificity of the Auszyme Monoclonal assay results by performing the study under conditions that minimized sample cross-contamination and by using additional HBV marker verification of positive samples.

In phase I of this study, all specimens were from females and were B-human chorionic gonadotropin (B-hCG)-positive sera or plasma specimens at the reference laboratory, had a volume of 2 ml or greater, and had not passed through a viral accessioning or testing area. The reference laboratory (Quest Diagnostics, Teterboro, N.J.) aliquoted each sample from the main specimen tube, marked each sample vial with the qualitative or quantitative B-hCG result and a unique identifier number, and shipped the samples by overnight delivery to Abbott Laboratories.

The Auszyme Monoclonal assay was performed on all samples in accordance with procedure C (incubation at 40°C for 75 min) of the package insert. Initially reactive samples were tested again in duplicate. If neither of the repeat tests was reactive, the specimen was considered negative for HBsAg. If either retest was reactive, the sample was considered repeatedly reactive (RR) and was then tested by the Auszyme confirmatory assay through procedure A. Only those specimens for which RR results were neutralized by the confirmatory procedure were considered positive for HBsAg (HBsAg confirmatory assay package insert [dated 1995], Abbott Laboratories Diagnostics Division, Abbott Park, Ill.).

These confirmed HBsAg-positive specimens were then tested by two additional licensed HBsAg assays: the IMx HBsAg assay (Abbott Laboratories), an automated microparticle-based assay with a monoclonal antibody capture phase and an enzyme-linked polyclonal antibody detection phase, and the Ortho Antibody to HBsAg ELISA Test System 2 (Ortho-Clinical Diagnostics, Raritan, N.J.), a microtiter assay using monoclonal antibody capture on the solid phase and an enzyme-linked monoclonal antibody detection phase. Additional tests were performed according to the manufacturer's package insert when there was sufficient sample volume. These tests included the CORAB radioimmunoassay (Abbott Laboratories), which detects HBV core protein-specific antibody; the HBe EIA (Abbott Laboratories), which detects HBeAg; and an in-house research assay for HBV DNA that uses nested PCR.

Phase II of the study was carried out by Abbott Laboratories and the Laboratory Corporation of America (LabCorp, Elmhurst, Ill.) reference laboratory. B-hCG-positive specimens provided by New York Biologics, Inc. (New York, N.Y.), were collected using the same criteria employed in phase I of this study, along with a signed patient informed-consent form. Aliquots of the same sample were shipped in parallel both to Abbott Laboratories and to the LabCorp reference laboratory, where the Auszyme Monoclonal assay was performed on all samples. Any initially reactive, RR, or confirmed-reactive sample identified at LabCorp was then tested at Abbott Laboratories using the pristine parallel sample. Discordant samples between the two sites were subjected to the testing described for phase I above. New York Biologics requested that samples showing a low-level reaction, i.e. an Auszyme sample-to-cutoff ratio between 1 and 2, be redrawn from the patients. The redrawn samples were evaluated in the same manner as the initial samples.

A population size of 1,286 would be needed to statistically validate an assay showing a 0.4% rate of prenatal HBV infection, which is the lowest rate reported by the CDC (upper and lower limits of 0.0067 and 0.0021, respectively, at a confidence level of 95% with binomial distribution). In case the rates of prenatal HBV infection had dropped significantly since the original CDC report in 1994, we chose to assume the possibility

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TABLE 1. RR, confirmed prenatal specimens from study phases I and II

Study phase (no. of samples tested)	No. (%) of samples		
	RR	Confirmed	Confirmed by additional test
I (4,988)	34 (0.68)	31 (0.62)	31 (0.62)
II (1,998)			
LabCorp reference lab	9 (0.45)	9 (0.45)	ND[a]
Abbott Laboratories	9 (0.45)	8 (0.40)[b]	7 (0.35)[c]
Total (6,986)	43 (0.62)	40 (0.57), 39 (0.56)[b]	38 (0.54)[c]

[a] ND, not done.
[b] Insufficient sample volume forced use of a 1:25 dilution for unconfirmed specimens. The second draw was CORAB (anticore) positive.
[c] Insufficient sample volume prevented testing of two of nine specimens from phase II.

of a 10-fold-lower infection prevalence of 0.04% to establish the number of specimens needed for the study. The targeted study population sizes of 5,000 for phase I and 2,000 for phase II would represent the midpoint of the population size required to validate a prenatal HBV infection rate of 0.04% (6,762 specimens at the 95% confidence level with binomial distribution).

Auszyme Monoclonal assay specificity was determined using the following formula: specificity = (number of true-negative specimens/[number of true-negative specimens + number of false-positive specimens]) × 100.

During phase I of the study, 4,988 specimens were received and tested. Of these samples, 34 (0.68%) were RR specimens and 31 of the 34 (0.62% of the total sample population) were neutralized in the confirmatory assay and considered positive for HBsAg. All 31 of the Auszyme Monoclonal HBsAg-positive specimens were reactive by the IMx HBsAg assay. Thirty of these specimens were also either reactive by the Ortho Antibody to HBsAg ELISA Test System 2 or reactive by testing for another HBV marker. Therefore, all Auszyme Monoclonal HBsAg-positive specimens were confirmed by at least one additional HBV marker test. Table 1 summarizes the RR and confirmatory rates for the phase I study. The HBV marker profile for the phase I specimens is shown in Table 2. The rate of RR, nonconfirmed specimens was 0.06% (3 of 4,988 specimens).

In phase II of the study, 1,998 specimens were tested by the Auszyme Monoclonal assay at the LabCorp reference laboratory. The rates of RR and confirmed-positive specimens for this part of the study are shown in Table 1. Nine specimens were RR at LabCorp. All nine samples were RR again when they were tested at Abbott Laboratories, indicating an RR rate of 0.45%. Of these nine RR specimens, one specimen was RR and confirmed to be positive at LabCorp but was not confirmed to be a neat specimen at Abbott. This sample had to be tested in the HBsAg confirmatory assay at a 1:25 dilution, due to insufficient sample volume, which may provide an explanation for the lack of confirmation. A second sample was redrawn from this patient 4 months after the initial sample collection. The Auszyme Monoclonal assay result was negative for the redrawn specimen, while the CORAB result was positive, suggesting that the original specimen was truly HBsAg positive.

Of the 6,986 prenatal specimens from both phases of the study, the overall rate of RR specimens was 0.62% (43 of 6,986 specimens) and the rate of RR and confirmed-positive specimens was 0.56% (39 of 6,986 specimens) (Table 1). Under these conditions, the specificity of the Auszyme Monoclonal assay was 99.96% [6,947 of (6,947 + 3) specimens], with a 95% confidence interval of 99.87 to 99.99% by binomial distribution.

Licensed HBsAg tests have a very high specificity and sensitivity if reactive tests are repeated and confirmed by neutralization, as is recommended by the manufacturers. However, non-RR or unconfirmed (nonneutralized)-reactive tests do occur (5). There are a number of possible reasons for such results. First, the patient may have an immune response to a vaccine (8). Second, there might be positive interference in the mouse monoclonal antibody-based immunoassays caused by the presence of human anti-mouse antibodies. The most common cause of human anti-mouse antibody interference is prior use of mouse monoclonal antibodies for therapeutic or imaging purposes (8). Third, a technician may perform a laboratory technique improperly, such as washing beads incorrectly or failing to remove particulate matter from specimens prior to testing (Auszyme Monoclonal package insert [dated 1995], Abbott Laboratories Diagnostics Division). Fourth, truly cross-reactive endogenous proteins may be present in the specimen.

Neutralized, RR specimens that do not correlate with the negative disease status of a patient exist. In these cases, nonreactive specimens may be contaminated by the transfer of antigen from positive samples, either through soiled equipment or the aerosolized transfer of antigen (10) (Auszyme Monoclonal package insert [dated 1995] and unpublished data, Abbott Laboratories Diagnostics Division). The proportion of high-risk individuals in a previously reported study population might have increased the likelihood of sample cross-contamination, resulting in an unusually high rate of false-positive test results with the Auszyme Monoclonal assay (11). In rare cases transient (neutralizable) antigenemia has been reported for both adults and infants postvaccination with HBV vaccine (4, 7). In these cases, assays detect residual vaccine.

Due to the above-listed factors, there is some concern that routine prenatal testing may lead to an increase in the number of enzyme immunoassay false-positive results in what is perceived as a low-risk population.

This study was designed to determine the rate of HBV infection in a general pregnant population and to evaluate the suitability of the Auszyme Monoclonal assay for widespread prenatal HBV screening. The approach was to use specimens that had never passed through a viral testing laboratory area

TABLE 2. Detailed HBV test results for RR, prenatal specimens from study phase I (n = 34)

Sample	Amt of B-hCG[a] (IU/liter)	Test sample by:						
		Auszyme Monoclonal assay	Confirmatory antibody neutralization	IMx HBsAg assay	CORAB assay	Abbott HBe EIA (HBeAg results)	Ortho Antibody to HBsAg ELISA Test System 2	Nested PCR
A162	1,407	+	+	+	−	−	+	+
A318	45	+	+	+	−	−	SMP QNS[b]	+
A658	200,492	+	+	+	+	−	+	+
A782	+	+	+	+	+	+	+	+
A785	977	+	+	+	−	−	+	+
A853	344	+	+	+	+	−	+	+
A906	303	+	+	+	+	−	+	+
A943	167,495	+	+	+	+	−	+	+
B085	+	+	+	+	+	−	+	+
B133	+	+	+	+	+	−	+	+
B142	4,730	+	+	+	+	−	+	+
B173	+	+	+	+	+	−	+	+
B532	+	+	+	+	+	−	+	−
B537	+	+	+	+	+	−	+	+
B647	104	+	+	+	+	−	+	+
C100	+	+	+	+	+	−	+	−
C123	161,824	+	+	+	+	−	+	−
C248	36	+	+	+	+	−	+	+
C502	485	+	−	−	−	−	−	−
C557	5,766	+	+	+	+	−	−	SMP QNS
C807	2,411	+	+	+	+	−	+	+
C835	9,101	+	+	+	+	+	+	+
C836	408	+	+	+	−	−	+	+
D757	5,937	+	+	+	−	−	SMP QNS	SMP QNS
D917	533	+	+	+	+	−	+	+
E028	35	+	+	+	+	−	+	−
E117	+	+	+	+	+	−	+	+
E197	1,526	+	−	SMP QNS	SMP QNS	SMP QNS	SMP QNS	+
E349	162,506	+	+	+	+	−	+	+
E436	271	+	+	+	+	−	+	−
E608	3,683	+	+	+	+	−	+	+
E644	7,242	+	+	+	+	−	+	−
E651	20,724	+	−	−	−	−	−	−
E779	+	+	+	+	+	−	+	+

[a] Quantitative or qualitative results as provided by the reference laboratory.
[b] SMP QNS, sample quantity not sufficient.

and were not likely to have been exposed to cross-contamination by HBV-reactive samples. Moreover, several independent markers for HBV were determined for the HBsAg-reactive specimens to provide a profile of the infection stage (6, 9). Finally, in phase II of this study, the positive samples were evaluated in parallel by two separate testing sites, which yielded equivalent enzyme immunoassay results.

The results reported here for the Auszyme Monoclonal assay indicate an overall RR rate of 0.62% and an RR, confirmed rate (determined as recommended by the manufacturer) of 0.56%, with an RR, nonconfirmed rate of only 0.04%. All of the RR, confirmed-positive specimens that had sufficient volume to undergo any further testing were confirmed to be truly positive by at least one additional test from a panel of tests that are indicative of viral load or different stages of HBV infection (6, 9). Based on these data, the specificity of the Auszyme Monoclonal assay is 99.96%. The overall HBsAg prevalence rate obtained in this study, 0.56%, is in excellent agreement with the 0.50% rate of positive HBsAg tests reported by the CDC when results from several studies involving pregnant women were averaged (1, 3, 5).

Three different HBsAg assay formats and multiple-marker verification of the RR, confirmed specimens suggest that there is no unique factor associated with pregnancy which led to an RR, confirmed specimen that was not truly positive.

In conclusion, in the present study, the rate of HBV infection in a total of 6,986 specimens obtained from pregnant females was 0.56%, a value that agrees well with prior CDC estimates for this population.

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